

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number  
**WO 01/05773 A1**

(51) International Patent Classification<sup>7</sup>: C07D 241/26,  
C07H 19/10, 19/207, A61K 31/495, 31/70, A61P 11/12

(21) International Application Number: PCT/US00/19775

(22) International Filing Date: 19 July 2000 (19.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/144,479 19 July 1999 (19.07.1999) US  
Not furnished 19 July 2000 (19.07.2000) US

(71) Applicant (for all designated States except US): THE  
UNIVERSITY OF NORTH CAROLINA AT CHAPEL  
HILL [US/US]; 308 Bynum Hall, Campus Box 4105,  
Chapel Hill, NC 27599-4105 (US).

(71) Applicant and

(72) Inventor: BOUCHER, Richard, C., Jr. [US/US]; 735  
Gimghoul Road, Chapel Hill, NC 27514 (US).

(74) Agent: MYERS BIGEL SIBLEY SAJOVEC, P.A.; Post  
Office Box 37428, Raleigh, NC 27627 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published:**

- With international search report.
- Before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments.

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: CONJUGATES OF SODIUM CHANNEL BLOCKERS AND METHODS OF USING THE SAME

(57) Abstract: Compounds of the general formula P<sub>1</sub>-L-P<sub>2</sub>; wherein "P<sub>1</sub>" is a pyrazinoylguanidine sodium channel blocker, "L" is a linking group, and "P<sub>2</sub>" is either (i) a pyrazinoylguanidine sodium channel blocker or (ii) a P2Y<sub>2</sub> receptor agonist, are disclosed. Pharmaceutical formulations containing the same and methods of use thereof to hydrate mucosal surfaces such as airway mucosal surfaces are also disclosed.

WO 01/05773 A1

## CONJUGATES OF SODIUM CHANNEL BLOCKERS AND METHODS OF USING THE SAME

### RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No.  
60/144,479, filed July 19, 1999, the disclosure of which is incorporated herein by  
5 reference in its entirety.

### STATEMENT OF FEDERAL SUPPORT

This invention was made with Government support under Grant No. HL51818  
10 from the National Institutes of Health. The United States government has certain  
rights in this invention.

### FIELD OF THE INVENTION

The present invention relates to conjugates of sodium channel blockers, and  
15 particularly covalent conjugates comprising a pyrazinoylguanidine sodium channel  
blocker and another compound such as another pyrazinoylguanidine sodium channel  
blocker or a P2Y<sub>2</sub> receptor agonist.

### BACKGROUND OF THE INVENTION

20 U.S. Patent No. 4,501,729 to Boucher describes the use of respirable or non-  
respirable amiloride to hydrate airway mucous secretions, and U.S. Patent No.  
5,656,256 to Boucher and Stutts describes the use of respirable or non-respirable  
benzamil and phenamil to hydrate lung mucus secretions. U.S. Patent No. 5,789,391  
to Jacobus describes methods of treating sinusitis by administering uridine  
25 triphosphates (UTP) and related compounds such as P<sup>1</sup>,P<sup>4</sup>-di(uridine-5'  
tetraphosphate (U<sub>2</sub>P<sub>4</sub>) in order to promote drainage of congested fluid in the sinuses.

U.S. Patent No. 5,292,498 to Boucher describes nucleotides, particularly P2Y<sub>2</sub>  
receptor agonists, that can be used to hydrate airway mucus secretions. Dinucleotides  
that can be used to hydrate airway mucus secretions are described in U.S. Patent No.  
30 5,635,160 to Stutts et al.. Additional compounds that are P2Y<sub>2</sub> receptor ligands and

can be used to hydrate airway mucus secretions are disclosed in W. Pendergast et al., U.S. Patent No. 5,837,861, along with U.S. Patents Nos. 5,763,447 to Jacobus and Leighton, and 5,789,391 to Jacobus et al.

### Summary of the Invention

5 A first aspect of the present invention is a compound (hereinafter also referred to as an "active compound" or "active agent") of the general formula  $P_1-L-P_2$ ; wherein " $P_1$ " is a pyrazinoylguanidine sodium channel blocker, "L" is a linking group, and " $P_2$ " is either (i) a pyrazinoylguanidine sodium channel blocker or (ii) a  
10  $P2Y_2$  receptor agonist. An advantage of compounds of the present invention is that they are substantially non-absorbable, or absorption-retardant or exhibit delayed absorption on mucosal (e.g., airway, gastrointestinal) surfaces, thereby contributing to a prolonged mode of action and fewer systemic side effects.

A second aspect of the present invention is a composition comprising an active  
15 compound as defined above in an effective therapeutic amount, in a pharmaceutically acceptable carrier.

A third aspect of the present invention is a method of treating a mucosal surface in a subject in need thereof, comprising administering an active compound as described herein in an amount effective to treat the subject. In general, treatment of  
20 the subject will mean that the mucosal surface being treated with a compound or composition of the present invention will be hydrated, or that the compound or composition being used will block or otherwise retard the absorption of liquid onto or onto the mucosal surface, or that the mucosal surface will otherwise exhibit an increased volume of liquid on the mucosal surface.

25 A fourth aspect of the present invention is the use of an active compound as described above for the preparation of a medicament for treating a mucosal surface in a subject in need thereof, as described herein.

The foregoing and other objects and aspects of the present invention are explained in detail in the specification set forth below.

30

### Brief Description of Drawings

FIG. 1 is a graphical illustration of the effect of apical washout of a compound of the present invention, where wash-out correlates with cellular uptake. A range of reversibilities is shown, with the compound CF-519 being completely reversible.

FIG. 2 is an example of a confocal microscopy assay of drug uptake into cultured airway epithelia. In this assay, a compound ( $10^{-4}$  M) is placed on the airway surface and fluorescence from the cells collected by x-z scanning confocal microscopy. The images shown on the left depict fluorescence in the cells 20 minutes after exposure to amiloride, benзамил and phenamil. Quantitation of the drug uptake is graphically illustrated on the right in terms of units of fluorescence.

### Detailed Description of the Invention

The present invention will now be described more fully hereinafter with reference to the accompanying figures, which further illustrate the invention described herein. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

The term "alkyl" or "loweralkyl" as used herein refers to C1 to C4, C6 or C8 alkyl, which may be linear or branched and saturated or unsaturated. Cycloalkyl is specified as such herein, and is typically C3, C4 or C5 to C6 or C8 cycloalkyl. Alkenyl or loweralkenyl as used herein likewise refers to C1 to C4 alkenyl, and alkoxy or loweralkoxy as used herein likewise refers to C1 to C4 alkoxy. The term "aryl" as used herein refers to C3 to C10 cyclic aromatic groups such as phenyl, naphthyl, and the like, and includes substituted aryl groups such as tolyl. "Halo" as used herein refers to any halogen group, such as chloro, fluoro, bromo, or iodo. The term "hydroxyalkyl" as used herein refers to C1 to C4 linear or branched hydroxy-substituted alkyl, i.e.,  $-\text{CH}_2\text{OH}$ ,  $-(\text{CH}_2)_2\text{OH}$ , etc. The term "aminoalkyl" as used herein refers to C1 to C4 linear or branched amino-substituted alkyl, wherein the term

"amino" refers to the group NR'R", wherein R' and R" are independently selected from H or lower alkyl as defined above, i.e., -NH<sub>2</sub>, -NHCH<sub>3</sub>, -N(CH<sub>3</sub>)<sub>2</sub>, etc. The term "oxyalkyl" as used herein refers to C1 to C4 oxygen-substituted alkyl, i.e., -OCH<sub>3</sub>, and the term "oxyaryl" as used herein refers to C3 to C10 oxygen-substituted cyclic aromatic groups.

The present invention is concerned primarily with the treatment of human subjects, but may also be employed for the treatment of other animal subjects (i.e., mammals, avians) for veterinary purposes. Mammals are preferred, with humans being particularly preferred.

The present invention is useful in treating mucosal surfaces in a subject in need of such treatment. "Treatment" includes the hydration of the mucosal surface, or the blocking or retardation of the absorption of liquid onto or into the mucosal surface, or an increase of volume of liquid on the mucosal surface, whether by increasing water or liquid on the mucosal surface, increasing the amount of salt on the surface, or both. In a preferred embodiment, the mucosal surface is an airway surface. The term "airway surface" as used herein refers to airway surfaces below the larynx and in the lungs (e.g., bronchial passages, alveolar passages), as well as air passages in the head, including the sinuses and other nasal airways, and in the region above the larynx. The present invention may also be used to treat mucosal surfaces other than airway surfaces. Such other mucosal surfaces include gastrointestinal surfaces, oral surfaces, genito-ureteral surfaces, ocular surfaces or surfaces of the eye, the inner ear, and the middle ear.

Subjects that may be treated by the methods of the present invention include patients afflicted with cystic fibrosis, primary ciliary dyskinesia, chronic bronchitis, chronic obstructive airway disease, artificially ventilated patients, patients with acute pneumonia, etc. Subjects that may be treated by the method of the present invention also include patients being nasally administered supplemental oxygen (which tends to dry the airway surfaces), patients afflicted with an allergic disease or response (e.g., an allergic response to pollen, dust, animal hair or particles, insects or insect particles, etc.) that affect nasal airway surfaces, patients afflicted with an infection caused by a microorganism (e.g., infections caused by such organisms as *Staphylococcus aureus*, *Haemophilus influenza*, *Streptococcus pneumoniae*, *Pseudomonas* spp. etc.) of the nasal airway surfaces, an inflammatory disease that affects nasal airway surfaces, or patients afflicted with sinusitis (wherein the active agent or agents are administered to

promote drainage of congested mucous secretions in the sinuses by administering an amount effective to promote drainage of congested fluid in the sinuses).

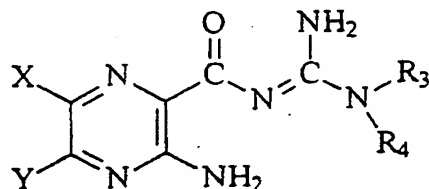
The compounds of the present invention can be prepared according to methods described herein, as well as in accordance with known techniques or variations thereof which will be apparent to skilled persons in light of the disclosure set forth herein. See, e.g., D. Benos et al., *Proc. Natl. Acad. Sci. USA* **83**, 8525 (1986); T. Kleyman et al., *Am. J. Physiol.* **250** (Cell Physiol. 19): C165-C170 (1986); U.S. Patent No. 3,313,813; U.S. Patent No. 4,501,729; U.S. Patent No. 5,789,391; U.S. Patent No. 5,292,498; U.S. Patent No. 5,635,160; U.S. Patent No. 5,837,861; U.S. Patents No. 5,763,447; and U.S. Patent No. 5,789,391 (the disclosures of all patent references cited herein are incorporated by reference in their entirety).

### 1. Sodium channel blockers.

Any sodium channel blocker (i.e.,  $P_1$  or  $P_2$  in the formula  $P_1-L-P_2$ ) can be used to carry out the present invention. Numerous pyrazinoylguanidine sodium channel blockers are disclosed in U.S. Patent No. 3,313,813 to Cragoe. Amiloride, one particular pyrazinoylguanidine sodium channel blocker, is described at Merck Index Registry No. 426 (12<sup>th</sup> Ed. 1996). Benzamil (also known as 3,5-diamino-6-chloro-N-(benzylaminoaminomethylene) pyrazinecarboxamide) and phenamil (also known as 3,5-diamino-6-chloro-N-(phenylaminoaminomethylene)pyrazinecarboxamide) are known compounds and are also disclosed in U.S. Pat. No. 3,313,813 to E. Cragoe.

Various additional pyrazinoylguanidine sodium channel blockers that are amiloride analogs are disclosed and described in T. Kleyman and E. Cragoe, *J. Membrane Biol.* **105**, 1-21 (1988).

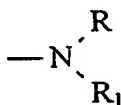
Preferred examples of active compounds that may be used to carry out the present invention are the pyrazinoylguanidine sodium channel blockers disclosed in U.S. Pat. No. 3,313,813, incorporated by reference above. Such compounds have the formula:



wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, Z-thio and Z-sulfonyl wherein Z is selected from the group consisting of loweralkyl and phenyl-loweralkyl. Preferably, X is chloro.

Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:  
wherein:



10

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

15 R<sub>1</sub> is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

R and R<sub>1</sub> can be joined to form a lower alkylene. Preferably, Y is amino.

R<sub>2</sub> is selected from the group consisting of hydrogen and loweralkyl.

20 Preferably, R, R<sub>1</sub>, and R<sub>2</sub> are hydrogen.

R<sub>3</sub> and R<sub>4</sub> are independently selected from the group consisting of hydrogen, loweralkyl, hydroxy-loweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl. Preferably, R<sub>3</sub> is hydrogen, phenyl, or phenylalkyl. Preferably, R<sub>4</sub> is hydrogen.

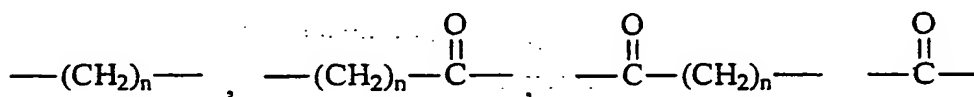
As discussed below, R<sub>4</sub> may be replaced with a linking group L.

## 2. Linking groups.

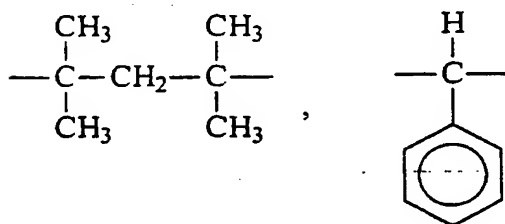
30 Any suitable linking group (*i.e.*, "L" in formula P<sub>1</sub>-L-P<sub>2</sub>) may be employed. The linking group may be a non-absorbable carrier moiety. The non-absorbable

carrier moiety may be a carbohydrate, protein, peptide, polyamine, or water soluble linear polymer. Water soluble linear polymers useful as carrier moieties include polyvinylpyrrolidone, polyethylene glycol, nonylphenol ethoxylates, and polyvinyl alcohol. Carbohydrates useful as carrier moieties include sugars and polysaccharides, such as dextran, lactose, and mannitol. An additional example is agarose. Proteins or peptides useful as carrier moieties include albumin (for example, human serum albumin) and protamine. Polyamines useful for carrying out the present invention include spermine and spermidine.

The linking groups may be the same as those groups set forth for  $R_4$  above, except that they are provided in divalent rather than univalent form. Linking groups may also be heteroatoms, such as -O-. Thus the linking group may be an alkylene, alkylencarbonyl, carbonylalkylene, or a carbonyl group, as follows:

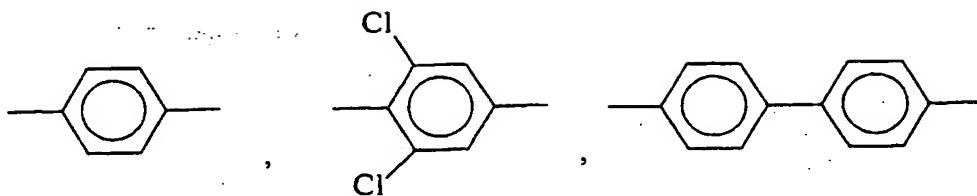


where  $n$  is 0 (*i.e.*, a direct covalent linkage) or is from 1 to 6. Such alkylene groups may be saturated or unsaturated, and may be substituted 1, 2, 3, or 4 times with C1-C4 alkyl, halo, phenyl, or halo-substituted phenyl. Examples are as follows:



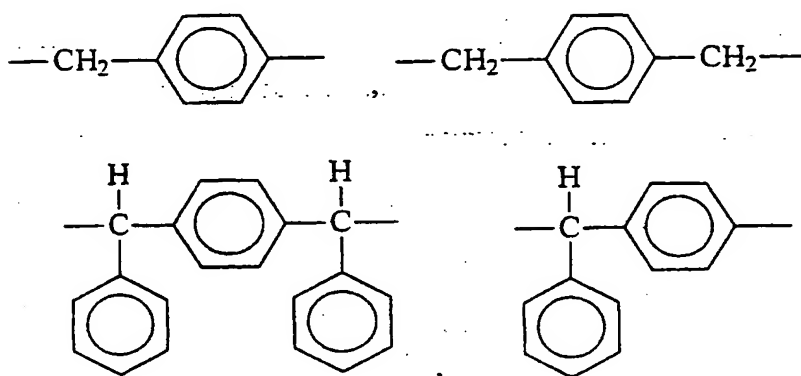
A phenyl or phenylene group, or two or more linked phenylene groups, may be provided as the linking group, which phenylene group may optionally be substituted 1, 2, 3 or four times with a halogen or alkyl group. Examples are as follows:



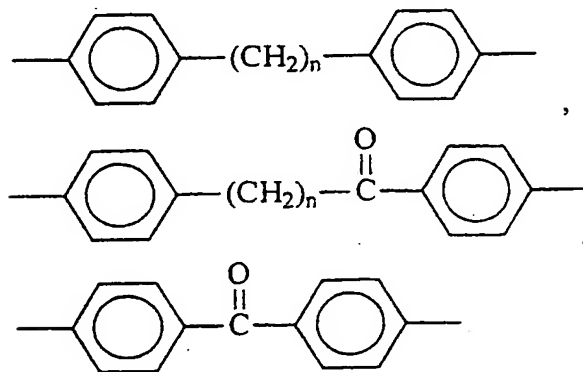


A substituted or unsubstituted phenylene group may be joined at either or both ends with a substituted or unsubstituted alkylene, alkylencarbonyl, carbonylalkylene, or carbonyl group as described above to provide a linking group. Examples are as

5 follows:

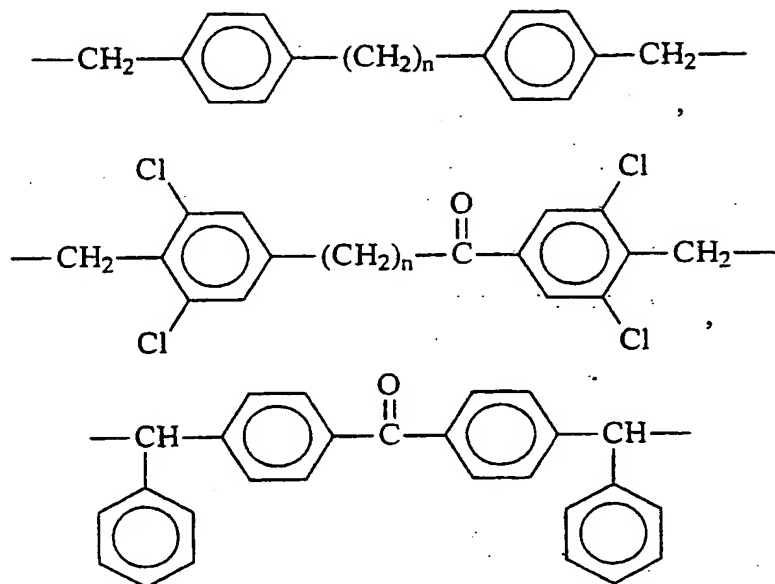


A substituted or unsubstituted alkylene, alkylencarbonyl, carbonylalkylene, or carbonyl group as described above may joined at either or both ends to a substituted or unsubstituted phenylene group as described above to provide a linking group. Examples are as follows:



where "n" is as defined above. Such compounds may be further substituted at either or both ends by a substituted or unsubstituted alkylene, alkylencarbonyl, carbonylalkylene, or carbonyl group, as described above, to provide still further linking groups. Examples are as follows:

5



where "n" is as defined above.

10

### 3. P<sub>2</sub>Y<sub>2</sub> Receptor agonists.

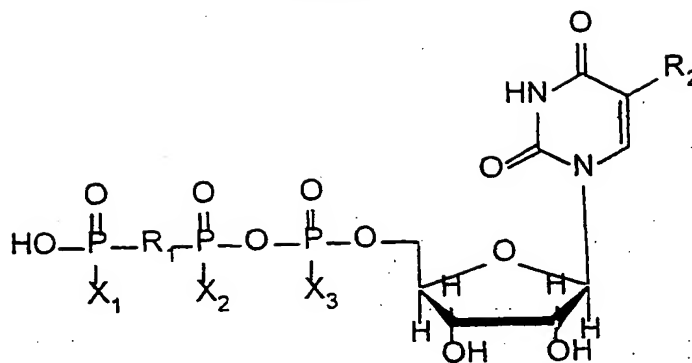
As noted above P<sub>2</sub> may also be a P<sub>2</sub>Y<sub>2</sub> receptor ligand, such as a nucleotide (e.g., ATP, UTP), dinucleotide (described in more detail hereinbelow), or derivative thereof. P<sub>2</sub>Y<sub>2</sub> receptor ligands that can be used to carry out the present invention include all of the compounds, particularly the nucleotides and dinucleotides that are

15 include all of the compounds, particularly the nucleotides and dinucleotides that are P<sub>2</sub>Y<sub>2</sub> ligands and are disclosed in W. Pendergast et al., U.S. Patent No. 5,837,861 (Nov. 17, 1998), along with all the compounds disclosed in U.S. Patents Nos. 5,763,447 to Jacobus and Leighton, 5,789,391 to Jacobus et al., 5,635,160 to Stutts et al., and 5,292,498 to Boucher, the disclosures of all of which are incorporated herein

20 by reference in their entirety.

Examples of such nucleotides are depicted in Formulae I - IV

Formula I



wherein:

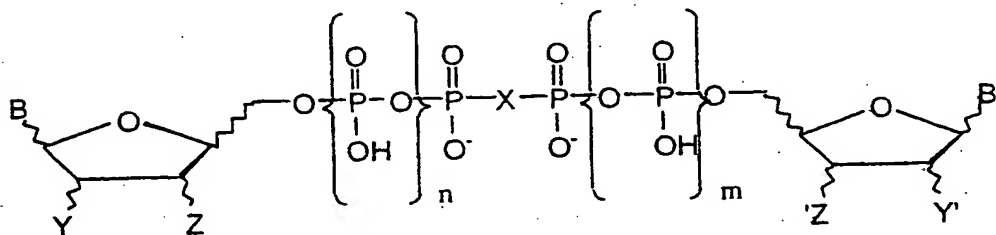
$X_1$ ,  $X_2$  and  $X_3$  are each independently either  $O^-$  or  $S^-$ ; preferably,  $X_2$  and  $X_3$  are  $O^-$ ;

$R_1$  is O, imido, methylene or dihalomethylene (e.g., dichloromethylene or difluoromethylene); preferably,  $R_1$  is oxygen or difluoromethylene;

$R_2$  is H or Br; preferably,  $R_2$  is H; particularly preferred compounds of Formula I are uridine 5'-triphosphate (UTP) and uridine 5'-O-(3-thiotriphosphate) (UTP<sub>γ</sub>S).

A dinucleotide is depicted by the general Formula II:

Formula II



15

wherein:

X is oxygen, methylene, difluoromethylene, imido;

$n = 0, 1$ , or  $2$ ;

$m = 0, 1$ , or  $2$ ;

20  $n + m = 0, 1, 2, 3$ , or  $4$ ; and

B and B' are each independently a purine residue or a pyrimidine residue linked through the 9- or 1- position, respectively;

Z = OH or  $N_3$ ;

Z' = OH or  $N_3$ ;

Y = H or OH;

Y' = H or OH;

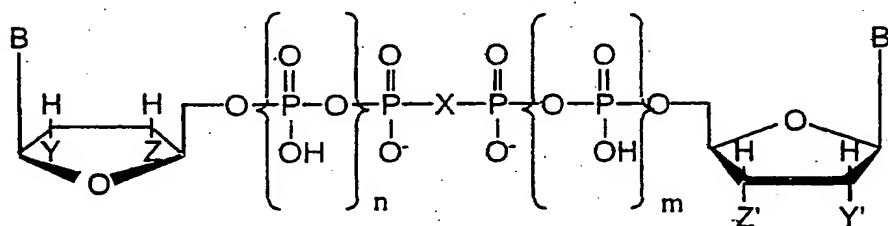
provided that when Z is N<sub>3</sub>, Y is H or when Z' is N<sub>3</sub>, Y' is H.

The furanose sugar is preferably in the β-configuration.

5 The furanose sugar is most preferably in the β-D-configuration.

Preferred compounds of Formula II are the compounds of Formula IIa:

Formula IIa



10

wherein:

X=O;

n+m=1 or 2;

Z, Z', Y, and Y'=OH;

15

B and B' are defined in Formulas IIc and IId;

X=O;

n+m=3 or 4;

Z, Z', Y, and Y'=OH;

20

B=uracil;

B' is defined in Formulas IIc and IId; or

X=O;

n+m=1 or 2;

25

Z, Y, and Y'=OH;

Z'=H;

B=uracil;

B' is defined in Formulas IIc and IId; or

30

X=O;

$n+m=0, 1, \text{ or } 2;$

$Z \text{ and } Y=\text{OH};$

$Z'=\text{N}_3;$

$Y'=\text{H};$

5  $\text{B}=\text{uracil};$

$\text{B}'=\text{thymine}; \text{ or}$

$\text{X}=\text{O};$

$n+m=0, 1, \text{ or } 2;$

10  $Z \text{ and } Z'=\text{N}_3;$

$Y \text{ and } Y'=\text{H};$

$\text{B} \text{ and } \text{B}'=\text{thymine}; \text{ or}$

$\text{X}=\text{CH}_2, \text{CF}_2, \text{ or } \text{NH};$

15  $n \text{ and } m=1;$

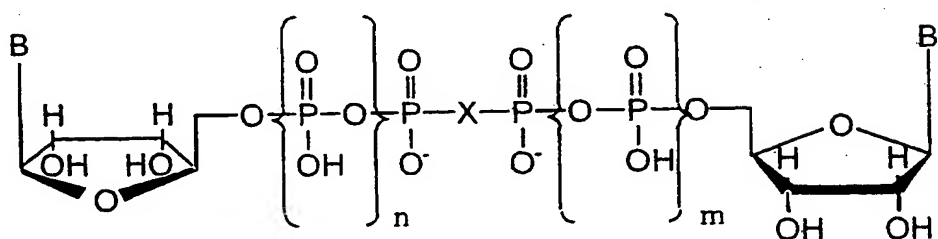
$Z, Z', Y, \text{ and } Y'=\text{OH};$

$\text{B} \text{ and } \text{B}' \text{ are defined in Formulas IIc and IId.}$

Another preferred group of the compounds of Formula II are the compounds of Formula IIb or the pharmaceutically acceptable salts thereof:

20

Formula IIb



25 wherein:

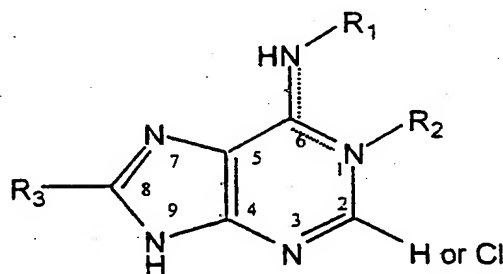
$\text{X}$  is oxygen, methylene, difluoromethylene, or imido;

$n = 0 \text{ or } 1;$

$m = 0 \text{ or } 1;$

$n + m = 0, 1, \text{ or } 2; \text{ and}$

B and B' are each independently a purine residue, as in Formula IIc, or a pyrimidine residue, as in Formula IId, linked through the 9- or 1- position, respectively. In the instance where B and B' are uracil, attached at N-1 position to the ribosyl moiety, then the total of m + n may equal 3 or 4 when X is oxygen. The ribosyl moieties are in the D- configuration, as shown, but may be L-, or D- and L-. The D- configuration is preferred.

Formula IIc

10

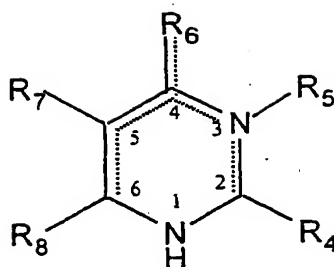
The substituted derivatives of adenine include adenine 1-oxide; 1,N<sup>6</sup>-(4- or 5-substituted etheno) adenine; 6-substituted adenine; or 8-substituted aminoadenine, where R' of the 6- or 8-HNR' groups are chosen from among: arylalkyl (C<sub>1-6</sub>) groups with the aryl moiety optionally functionalized as described below; alkyl; and alkyl groups with functional groups therein, such as: ([6-aminohexyl]carbamoylmethyl)-, and ω-acylated-amino(hydroxy, thiol and carboxy) derivatives where the acyl group is chosen from among, but not limited to, acetyl, trifluoroacetyl, benzoyl, substituted-benzoyl, etc., or the carboxylic moiety is present as its ester or amide derivative, for example, the ethyl or methyl ester or its methyl, ethyl or benzamido derivative. The ω-amino(hydroxy, thiol) moiety may be alkylated with a C<sub>1-4</sub> alkyl group.

20

Likewise, B or B' or both in Formula IIb may be a pyrimidine with the general formula of Figure IId, linked through the 1-position:

25

Figure IId



wherein:

R<sub>4</sub> is hydroxy, mercapto, amino, cyano, aralkoxy, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> alkylamino, and dialkylamino, the alkyl groups optionally linked to form a  
 5 heterocycle;

R<sub>5</sub> is hydrogen, acyl, C<sub>1-6</sub> alkyl, aroyl, C<sub>1-5</sub> alkanoyl, benzoyl, or sulphonate;

R<sub>6</sub> is hydroxy, mercapto, alkoxy, aralkoxy, C<sub>1-6</sub>-alkylthio, C<sub>1-5</sub> disubstituted amino, triazolyl, alkylamino, or dialkylamino, where the alkyl groups  
 10 are optionally linked to form a heterocycle or linked to N-3 to form an optionally substituted ring;

R<sub>7</sub> is hydrogen, hydroxy, cyano, nitro, alkenyl, with the alkenyl moiety optionally linked through oxygen to form a ring optionally substituted on the carbon adjacent to the oxygen with alkyl or aryl groups, substituted alkynyl or hydrogen  
 15 where R<sub>8</sub> is amino or substituted amino and halogen, alkyl, substituted alkyl, perhalomethyl (e.g., CF<sub>3</sub>), C<sub>2-6</sub> alkyl, C<sub>2-3</sub> alkenyl, or substituted ethenyl (e.g., allylamino, bromvinyl and ethyl propenoate, or propenoic acid), C<sub>2-3</sub> alkynyl or substituted alkynyl when R<sub>6</sub> is other than amino or substituted amino and together R<sub>5</sub> - R<sub>6</sub> may form a 5- or 6-membered saturated or unsaturated ring bonded through N or  
 20 O at R<sub>6</sub>, such a ring may contain substituents that themselves contain functionalities;

R<sub>8</sub> is hydrogen, alkoxy, arylalkoxy, alkylthio, arylalkylthio, carboxamidomethyl, carboxymethyl, methoxy, methylthio, phenoxy, or phenylthio.

In the general structure of Figure II d above, the dotted lines in the 2- to 6-- positions are intended to indicate the presence of single or double bonds in these  
 25 positions; the relative positions of the double or single bonds being determined by whether the R<sub>4</sub>, R<sub>6</sub>, and R<sub>7</sub> substituents are capable of keto-enol tautomerism.

In the general structures of Figure II c and II d above, the acyl groups advantageously comprise alkanoyl or aroyl groups. The alkyl groups advantageously contain 1 to 8 carbon atoms, particularly 1 to 4 carbon atoms optionally substituted by

one or more appropriate substituents, as described below. The aryl groups including the aryl moieties of such groups as aryloxy are preferably phenyl groups optionally substituted by one or more appropriate substituents, as described below. The above mentioned alkenyl and alkynyl groups advantageously contain 2 to 8 carbon atoms, particularly 2 to 6 carbon atoms, e.g., ethenyl or ethynyl, optionally substituted by one or more appropriate substituents as described below. Appropriate substituents on the above-mentioned alkyl, alkenyl, alkynyl, and aryl groups are advantageously selected from halogen, hydroxy, C<sub>1-4</sub> alkoxy, C<sub>1-4</sub> alkyl, C<sub>6-12</sub> arylalkoxy, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic, amino, and substituted amino wherein the amino is singly or doubly substituted by a C<sub>1-4</sub> alkyl, and when doubly substituted, the alkyl groups optionally being linked to form a heterocycle.

For purposes of further clarifying the foregoing descriptions of Formulae IIc and IId, the descriptions can be simplified to the following:

R<sub>2</sub> is O or is absent; or

R<sub>1</sub> and R<sub>2</sub> taken together may form optionally substituted 5-membered fused imidazole ring; or

R<sub>1</sub> of the 6-HNR<sub>1</sub> group or R<sub>3</sub> of the 8-HNR<sub>3</sub> group is chosen from the group consisting of:

(a) arylalkyl (C<sub>1-6</sub>) groups with the aryl moiety optionally substituted,

(b) alkyl,

(c) ([6-aminoethyl]carbamoylmethyl),

(d) ω-amino alkyl (C<sub>2-10</sub>),

(e) ω-hydroxy alkyl (C<sub>2-10</sub>),

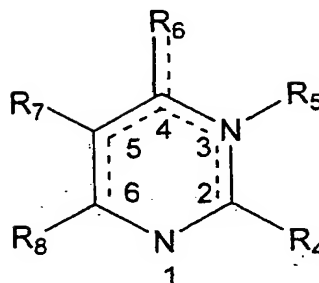
(f) ω-thiol alkyl (C<sub>2-10</sub>),

(g) ω-carboxy alkyl (C<sub>2-10</sub>),

(h) the ω-acylated derivatives of (b), (c) or (d) wherein the acyl group is either acetyl, trifluoroacetyl, benzoyl, or substituted-benzoyl alkyl(C<sub>2-10</sub>), and

(i) ω-carboxy alkyl (C<sub>2-10</sub>) as in (e) above wherein the carboxylic moiety is an ester or an amide;



Formula II d

wherein:

R<sub>4</sub> is hydroxy, mercapto, amino, cyano, aralkoxy, C<sub>1-6</sub> alkylthio, C<sub>1-6</sub> alkoxy, C<sub>1-6</sub> alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle;

R<sub>5</sub> is hydrogen, acyl, C<sub>1-6</sub> alkyl, aryl, C<sub>1-5</sub> alkanoyl, benzoyl, or sulphonate;

R<sub>6</sub> is hydroxy, mercapto, alkoxy, aralkoxy, C<sub>1-6</sub>-alkylthio, C<sub>1-5</sub> disubstituted amino, triazolyl, alkylamino or dialkylamino, wherein the alkyl groups of said dialkylamino are optionally linked to form a heterocycle or linked to N<sup>3</sup> to form an optionally substituted ring;

R<sub>5</sub> - R<sub>6</sub> together forms a 5 or 6-membered saturated or unsaturated ring bonded through N or O at R<sub>6</sub>, wherein said ring is optionally substituted;

R<sub>7</sub> is selected from the group consisting of:

- (a) hydrogen,
- (b) hydroxy,
- (c) cyano,
- (d) nitro,
- (e) alkenyl, wherein the alkenyl moiety is optionally linked through oxygen to form a ring optionally substituted with alkyl or aryl groups on the carbon adjacent to the oxygen,
- (f) substituted alkynyl
- (g) halogen,
- (h) alkyl,
- (i) substituted alkyl,
- (j) perhalomethyl,

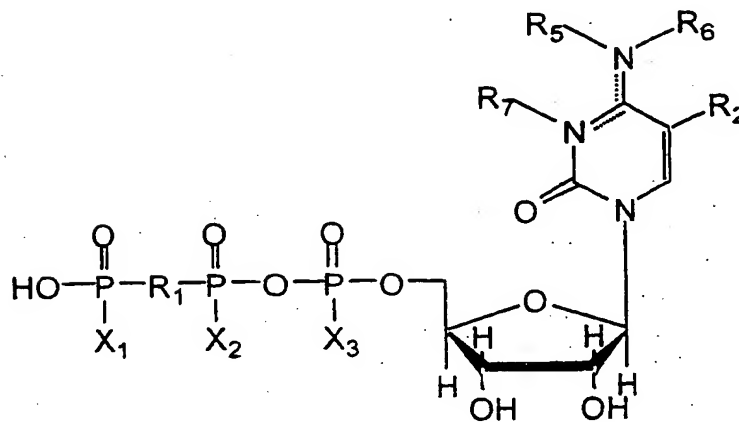
- (k) C<sub>2-6</sub> alkyl,  
(l) C<sub>2-3</sub> alkenyl,  
(m) substituted ethenyl,  
(n) C<sub>2-3</sub> alkynyl and  
(o) substituted alkynyl when R<sub>6</sub> is other than amino or substituted amino;

R<sub>8</sub> is selected from the group consisting of:

- (a) hydrogen,  
(b) alkoxy,  
(c) arylalkoxy,  
(d) alkylthio,  
(e) arylalkylthio,  
(f) carboxamidomethyl,  
(g) carboxymethyl,  
(h) methoxy,  
(i) methylthio,  
(j) phenoxy and  
(k) phenylthio.

CTP and its analogs are depicted by general Formula III:

Formula III



wherein:

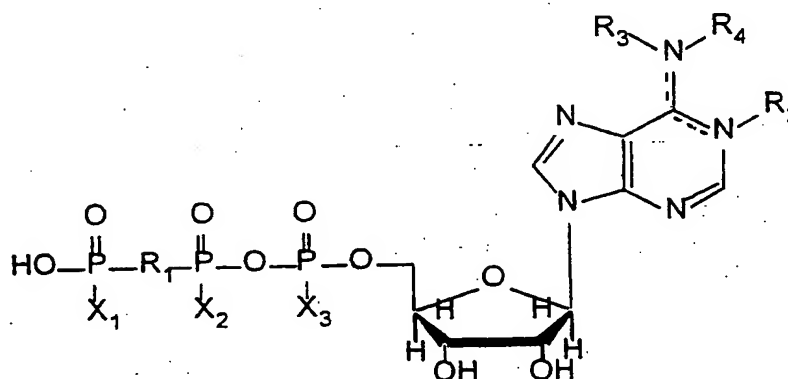
R<sub>1</sub>, X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> are defined as in Formula I;

$R_5$  and  $R_6$  are H while  $R_7$  is nothing and there is a double bond between N-3 and C-4 (cytosine), or

$R_5$ ,  $R_6$  and  $R_7$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-3 to N-4 with a double bond between N-4 and C-4 (3, $N^4$ -ethenocytosine) optionally substituted at the 4- or 5-position of the etheno ring.

ATP and its analogs are depicted by general Formula IV:

Formula IV



10

wherein:

$R_1$ ,  $X_1$ ,  $X_2$ , and  $X_3$  are defined as in Formula I;

$R_3$  and  $R_4$  are H while  $R_2$  is nothing and there is a double bond between N-1 and C-6 (adenine), or

$R_3$  and  $R_4$  are H while  $R_2$  is O and there is a double bond between N-1 and C-6 (adenine 1-oxide), or

$R_3$ ,  $R_4$ , and  $R_2$  taken together are  $-\text{CH}=\text{CH}-$ , forming a ring from N-6 to N-1 with a double bond between N-6 and C-6 (1, $N^6$ -ethenoadenine).

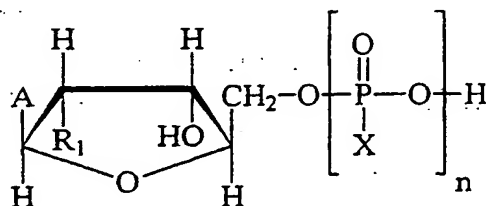
For simplicity, Formulas I, II, III, and IV herein illustrate the active compounds in the naturally occurring D-configuration, but the present invention also encompasses compounds in the L-configuration, and mixtures of compounds in the D- and L-configurations, unless otherwise specified. The naturally occurring D-configuration is preferred.

Some compounds of Formulas I, II, III, and IV can be made by methods which are well known to those skilled in the art and in accordance with known procedures (Zamecnik, P., *et al.*, *Proc. Natl Acad. Sci. USA* 89:2370-2373 (1992); Ng, K., *et al.*, *Nucleic Acids Res.* 15:3572-3580 (1977); Jacobus, K.M., *et al.*, U.S. Patent No.

5,789,391 and Pendergast, W., *et al.*, International Patent Application WO98/34942)); some are commercially available, for example, from Sigma Chemical Company, PO Box 14508, St. Louis, MO 63178. The synthetic methods of U.S. Patent 5,789,391 and International Patent Application WO98/34942 are incorporated herein by  
 5 reference in their entirety.

Thus, examples of compounds that can be used to carry out the present invention include compounds of Formula I-IV above, and include compounds having the general formula:

10



15 wherein:

X may be O or S;

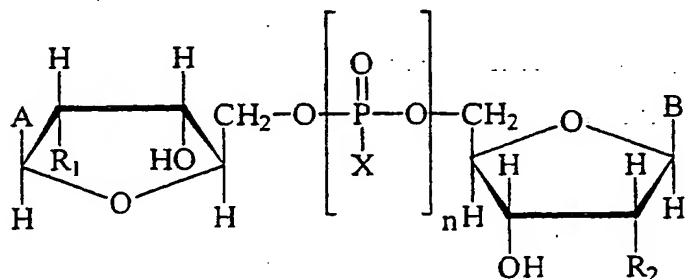
A is a purine or pyrimidine base (*e.g.*, adenine, guanine, thymine, cytosine, uracil)(each purine or pyrimidine base is preferably joined to the ribose or deoxyribose ring by covalent bond to the 9 nitrogen in the case of purines, or by  
 20 covalent bond to the 1 nitrogen in the case of pyrimidines);

R<sub>1</sub> is H or OH; and

n is from 1 to 4 or 6, preferably 2, 3 or 4.

Additional examples of receptor agonists that can be used to carry out the present invention are dinucleotides, including those having the general formula:

25



30

wherein:

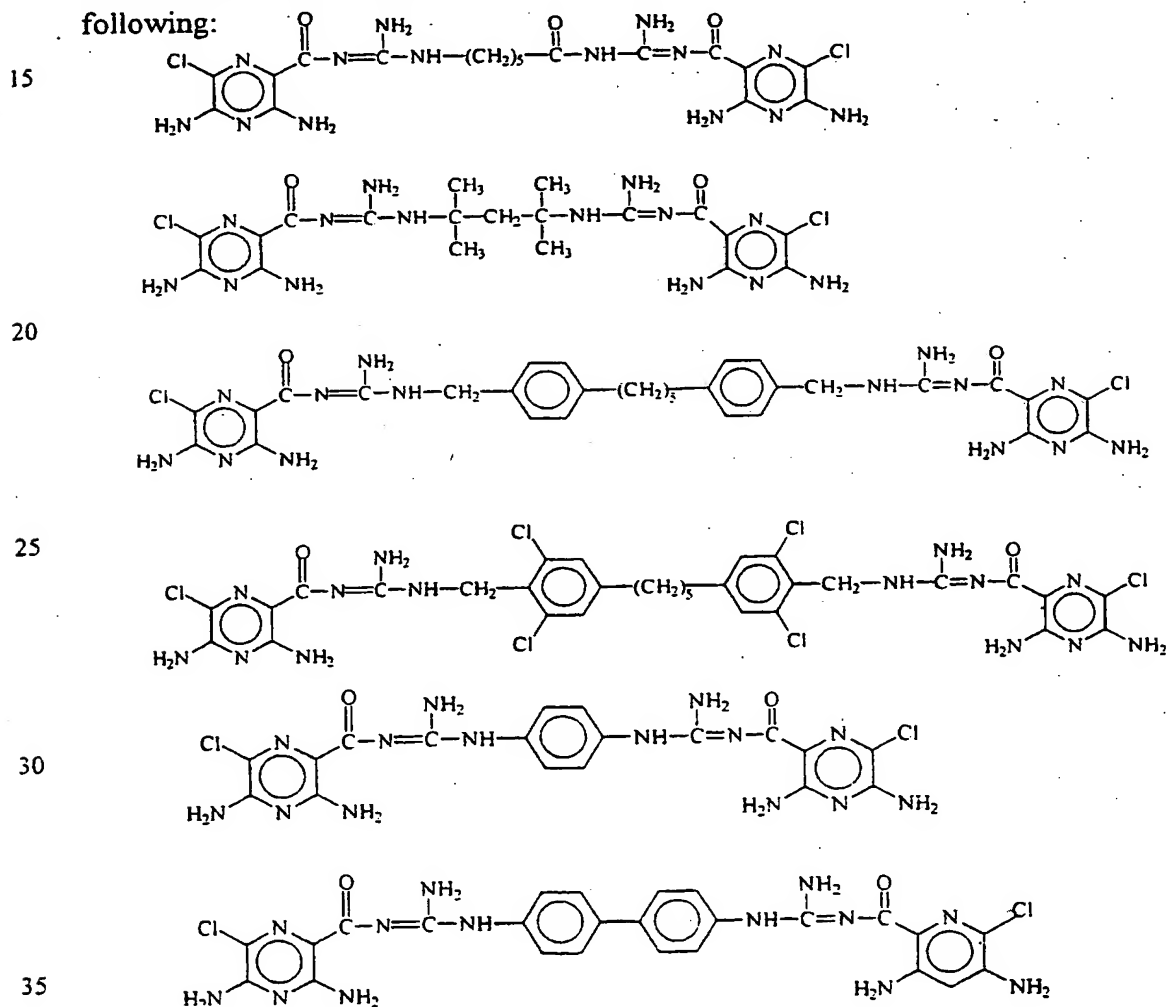
A and B are each independently a purine or pyrimidine base (*e.g.*, adenine, guanine, thymine, cytosine, uracil); preferably, A is uracil and B is cytosine;

$R_1$  and  $R_2$  are each independently selected from the group consisting of H or OH; and  $n$  is from 1 to 6, preferably 3 or 4.

For P2Y<sub>2</sub> receptor ligands as described herein, the linking group may be covalently joined to the purine or pyrimidine base, or the corresponding ribose or deoxyribose ring (e.g., of the compounds of Formula I-IV above), or attached to the terminal phosphate moiety of compounds represented by Formulae I, II and IV above, by any suitable means, such as by covalently joining the linking group thereto in any suitable position (e.g., a ring carbon such as the 5 carbon in a pyrimidine, or the 2, 6 or 8 carbon in a purine), to which linking group the ligand may be covalently attached.

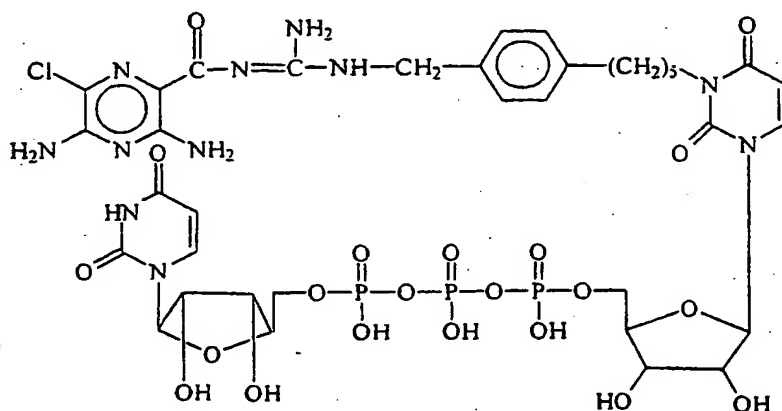
#### 4. Example conjugate compounds.

Specific examples of active compounds of the present invention, where P<sub>2</sub> is a pyrazinoylguanidine sodium channel blocker, include but are not limited to the following:



Additional examples of conjugate compounds useful in the present invention include those compounds whose structures are shown in Table 1, below, and in the Examples that follow.

Examples of active compounds of the present invention, where P<sub>2</sub> is a P2Y<sub>2</sub> receptor ligand, are as follows:



## 5. Pharmaceutically acceptable salts.

The term "active agent" as used herein, includes the pharmaceutically acceptable salts of the compound, such as (but not limited to) benzamil hydrochloride or phenamil hydrochloride. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, palmitic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acid, methanesulfonic acid, p-toluenesulfonic acid, naphthalenedisulfonic acid, polygalacturonic acid, and the like; and (b) salts formed from elemental anions such as chlorine, bromine, and iodine.

For nucleotides or dinucleotide active compounds, the compounds may be prepared as an alkali metal salt such as sodium or potassium, an alkaline earth metal salt, or an ammonium and tetraalkyl ammonium salt, NX<sub>4</sub><sup>+</sup> (wherein X is a C<sub>1-4</sub>) alkyl

group. Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects.

Active agents used to prepare compositions for the present invention may alternatively be in the form of a pharmaceutically acceptable free base of active agent.

5 Because the free base of the compound is less soluble than the salt, free base compositions are employed to provide more sustained release of active agent to the lungs. Active agent present in the lungs in particulate form which has not gone into solution is not available to induce a physiological response, but serves as a depot of bioavailable drug which gradually goes into solution.

10

## 6. Formulations and administration.

A third aspect of the present invention is a pharmaceutical formulation, comprising an active compound as described above in a pharmaceutically acceptable carrier (e.g., an aqueous carrier solution). In general, the active compound is included  
15 in the composition in an amount effective to treat mucosal surfaces, such as inhibit the reabsorption of water by airway surfaces, including nasal airway surfaces.

The active compounds disclosed herein may be administered to mucosal surfaces by any suitable means, including topically, parenterally (e.g., by intravenous, intramuscular, or intraperitoneal injection), orally, rectally, via  
20 inhalation, transdermally, etc. For example, for the treatment of constipation, the active compounds may be administered orally or rectally to the gastrointestinal mucosal surface. The active compound may be combined with a pharmaceutically acceptable carrier in any suitable form, such as sterile physiological saline for an injectable or topical solution, as a droplet, tablet or the like for oral administration, as  
25 a suppository for rectal or genito-ureteral administration, etc. Excipients may be included in the formulation to enhance the solubility of the active compounds, as desired.

The active compounds disclosed herein may be administered to the airway surfaces of a patient by any suitable means, including as a spray, mist, or droplets of  
30 the active compounds in a pharmaceutically acceptable carrier such as physiological saline solution or distilled water. For example, the active compounds may be prepared as formulations and administered as described in U.S. Patent No. 5,789,391 to Jacobus, the disclosure of which is incorporated by reference herein in its entirety.

In one preferred embodiment they are administered by administering an aerosol suspension of respirable or non-respirable particles (preferably non-respirable particles) comprised of the active compound, which the subject inhales through the nose. The respirable or non-respirable particles may be liquid or solid. The quantity of active agent included may be an amount sufficient to achieve dissolved concentrations of active agent on the airway surfaces of the subject of from about  $10^{-9}$ ,  $10^{-8}$ , or  $10^{-7}$  to about  $10^{-3}$ ,  $10^{-2}$ , or  $10^{-1}$  Moles/liter, and more preferably from about  $10^{-6}$  to about  $10^{-4}$  Moles/liter.

In one embodiment of the invention, the particulate active agent composition may contain both a free base of active agent and a pharmaceutically acceptable salt such as benzamil hydrochloride or phenamil hydrochloride to provide both early release of and sustained release of active agent for dissolution into the mucous secretions of the nose. Such a composition serves to provide both early relief to the patient, and sustained relief over time. Sustained relief, by decreasing the number of daily administrations required, is expected to increase patient compliance with a course of active agent treatments.

Solid or liquid particulate active agent prepared for practicing the present invention should as noted above include particles of respirable or non-respirable size: that is, for respirable particles, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs, and for nonrespirable particles, particles sufficiently large to be retained in the nasal airway passages rather than pass through the larynx and into the bronchi and alveoli of the lungs. In general, particles ranging from about 1 to 5 microns in size (more particularly, less than about 4.7 microns in size) are respirable. Particles of non-respirable size are greater than about 5 microns in size, up to the size of visible droplets. Thus, for nasal administration, a particle size in the range of 10-500  $\mu\text{m}$  may be used to ensure retention in the nasal cavity.

The dosage of active compound will vary depending on the condition being treated and the state of the subject, but generally may be an amount sufficient to achieve dissolved concentrations of active compound on the nasal airway surfaces of the subject of from about  $10^{-9}$ ,  $10^{-8}$ , or  $10^{-7}$  to about  $10^{-3}$ ,  $10^{-2}$ , or  $10^{-1}$  Moles/liter, and more preferably from about  $10^{-6}$  to about  $3 \times 10^{-4}$  Moles/liter. Depending upon the solubility of the particular formulation of active compound administered, the daily



dose may be divided among one or several unit dose administrations. The daily dose by weight may range from about .1, .5 or 1 to 10 or 20 milligrams of active agent particles for a human subject, depending upon the age and condition of the subject. A currently preferred unit dose is about .005 milligrams of active agent given at a regimen of four administrations per day. The dosage may be provided as a prepackaged unit by any suitable means (e.g., encapsulating in a gelatin capsule).

Pharmaceutical formulations suitable for airway administration include formulations of solutions, emulsions, suspensions and extracts. *See generally*, J. Nairn, *Solutions, Emulsions, Suspensions and Extracts*, in *Remington: The Science and Practice of Pharmacy*, chap. 86 (19<sup>th</sup> ed 1995). Pharmaceutical formulations suitable for nasal administration may be prepared as described in U.S. Patents Nos. 4,389,393 to Schor; 5,707,644 to Illum; 4,294,829 to Suzuki; and 4,835,142 to Suzuki; the disclosures of which are incorporated by reference herein in their entirety.

In the manufacture of a formulation according to the invention, active agents or the physiologically acceptable salts or free bases thereof are typically admixed with, *inter alia*, an acceptable carrier. The carrier must, of course, be acceptable in the sense of being compatible with any other ingredients in the formulation and must not be deleterious to the patient. The carrier may be a solid or a liquid, or both, and is preferably formulated with the compound as a unit-dose formulation, for example, a capsule, which may contain from 0.5% to 99% by weight of the active compound. One or more active compounds may be incorporated in the formulations of the invention, which formulations may be prepared by any of the well-known techniques of pharmacy consisting essentially of admixing the components.

Mists or aerosols of liquid particles comprising the active compound may be produced by any suitable means, such as by a simple nasal spray with the active agent in an aqueous pharmaceutically acceptable carrier, such as sterile saline solution or sterile water. Administration may be with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer. *See, e.g.*, U.S. Pat. No. 4,501,729 and 5,656,256. Suitable formulations for use in a nasal droplet or spray bottle or in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. The carrier is typically water (and most preferably sterile, pyrogen-free water) or a dilute aqueous alcoholic solution, preferably made isotonic with body fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not

made sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

Mists or aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator.

5 Aerosol generators for administering solid particulate medicaments to a subject produce particles which are respirable or non-respirable, as explained above, and generate a volume of mist or aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for administration  
10 by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder  
15 delivered by air drawn through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of  
20 illustrative aerosol generator comprises a metered dose inhaler. Metered dose inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 150 or 200  $\mu$ l to produce a fine particle spray containing the  
25 active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants (such as oleic acid or sorbitan trioleate), antioxidants and suitable flavoring agents.

30 Compositions containing respirable or non-respirable dry particles of micronized active agent may be prepared by grinding the dry active agent with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates.

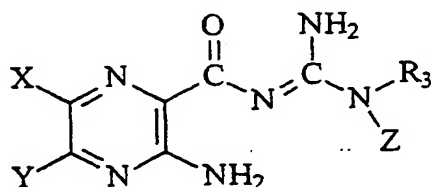
The particulate active agent composition may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the active agent in any suitable ratio (e.g., a 1 to 1 ratio by weight).

5

**7. Covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety.**

In addition to the foregoing, the pyrazinoylguanidine sodium channel blockers described above can be conjugated to a non-absorbable carrier moiety as described above to provide compounds that are active in hydrating mucosal surfaces. Some of these compounds have the formula:

10



wherein: X, Y, R<sub>2</sub> and R<sub>3</sub> are as defined above, and Z is a non-absorbable carrier moiety as described above covalently linked to the adjacent nitrogen atom; or a pharmaceutically acceptable salt thereof. Such compounds can be prepared, formulated and administered in essentially the same manner as described above for the same uses as described above.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof. In these Examples, Proton NMR spectra (360 MHz) and carbon NMR spectra (90 MHz) were obtained on a Bruker WM-360 spectrometer using tetramethylsilane as an internal standard. Liquid chromatography (LC)/mass spectroscopy (MS) was performed on a Perkin Elmer Sciex API 100 by one of the following methods:

20

Method A: YMC Pro C8 column, 5 $\mu$ , 150 x 4.6 mm; Mobile phase A = water + 0.4% acetic acid, B = acetonitrile (MeCN) + 0.4% acetic acid; Gradient: 5% B for 1 min, to 80% B in 7 min, followed by 100% B for 5 min.

25

Method B: YMC Pro C8 column, 5 $\mu$ , 150 x 4.6 mm; Mobile Phase A = water + 0.4% acetic acid, B = MeCN + 0.4% acetic acid; Gradient: 5% B for 1 min, going up to 80% B in 5 min.

Method C: Luna C8 (2) column, 150 x 4.6 mm, 5 $\mu$ , detector  $\lambda$  = 360 nm, mobile phase A = water + 0.4% acetic acid, B= MeCN + 0.4% acetic acid; Gradient: 5% B for 1 min, to 80% B in 7 min, followed by washout with 100% B for 5 min.

Analytical HPLC was performed on a Shimadzu HPLC 10Avp by one of the  
5 following methods:

Method D: Luna C18(2) column, 5 $\mu$ , 250 x 4.6 mm; detector  $\lambda$  = 360 nm; Gradient: A = water + 0.1% trifluoroacetic acid(TFA), B= MeCN + 0.1% TFA, concentration of MeCN increases from 10 to 60% during a 0-11 min interval, then 60-100% from 11-12 min.

10 Method E: Symmetry C8 column, 150 x 4.6 mm; detector  $\lambda$  = 360 nm; Gradient: A = water + 0.1% TFA, b= MeCN + 0.1% TFA, concentration of B increases in the A/B mixture from 10 to 60% during the 0-11 min interval, then B increases to 60-100% from 11-12 min.

Preparative HPLC was performed on a Gilson CombiChem by methods  
15 described in below in the Examples.

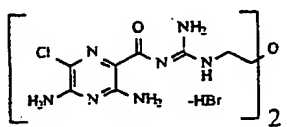
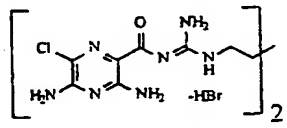
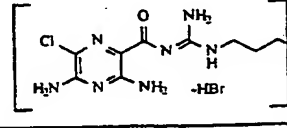
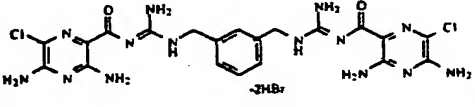
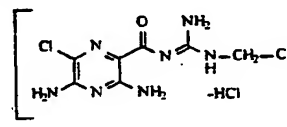
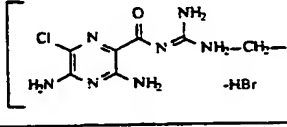
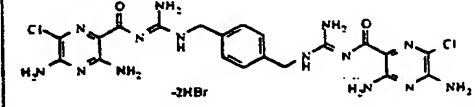
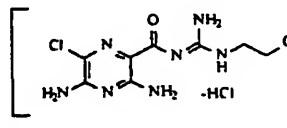
### EXAMPLE 1

#### Synthesis of Dimeric Compounds

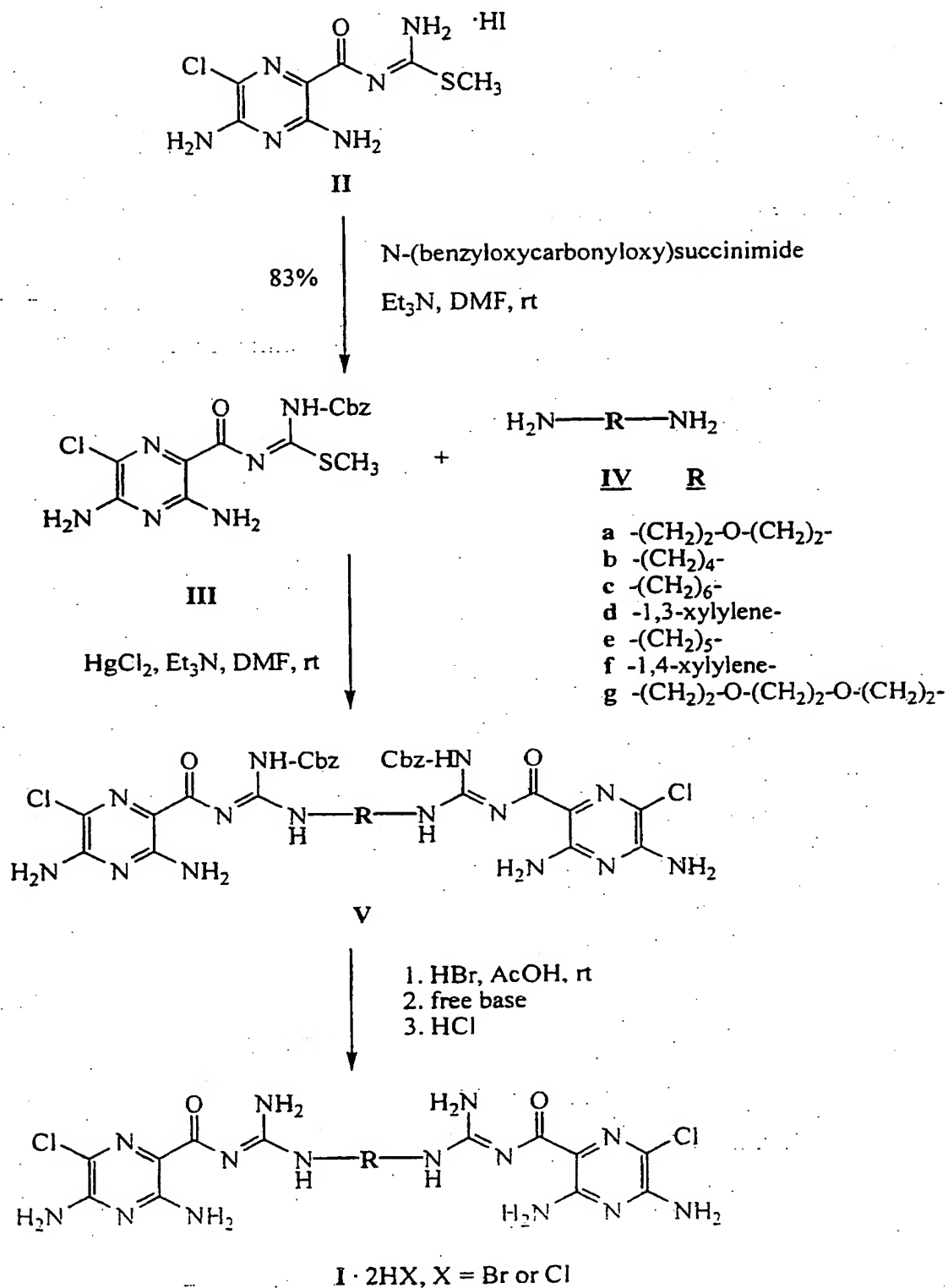
Referring to **Scheme 1**, dimer compounds of Formula **I** have been synthesized  
20 as shown in **TABLE 1**. The synthesis begins with 1-(3,5-diamino-6-chloropyrazinoyl)-2-methyl-2-thiopseudourea hydroiodide (intermediate **II**, prepared as described in U.S. Patent No. 4,246,406 to Cragoe et al).. Intermediate **II** was treated with N-(benzyloxycarbonyloxy)succinimide and triethylamine in N,N-dimethylformamide (DMF) to give the carbobenzyloxy(Cbz)-protected intermediate  
25 **III**. Formation of the Cbz-protected dimer **V** occurs upon treatment of intermediate **III** with the appropriate diamine **IV** in the presence of mercury(II) chloride and triethylamine in DMF (conditions reported by W. Su, *Synth. Comm.*, 26, 407-413 (1996) for the preparation of Cbz-protected guanidines). Treatment of dimer intermediate **V** with hydrobromic acid in acetic acid removes both of the Cbz-  
30 protecting groups to give amiloride dimer **I** as the dihydrobromide salt. The hydrobromide salts of **I** could be converted to the free base of **I** by treatment with a strong base like potassium hydroxide in aqueous media. The free base can then be converted to other salt forms (e.g., hydrochloride salt or other pharmaceutically acceptable salt forms) by treatment with the appropriate acid.

TABLE 1

## Dimers

Compound (AMR code)	Structure	IC <sub>50</sub> (nM)	Max Inhibition (%) from baseline	Effect of washout (3) 100% = Baseline
CF-509 1363		1275	79	77
CF-510 1390		81	73	21
CF-511 1438		114	86	54
CF-512 1465		197	92	60
CF-514 1504A		121	96	20
CF-515 1504		133	99	12
CF-516 1527		330	90	65
* CF-519 1604		1075	85	100

## Scheme 1



## EXAMPLES 2 Through 10

Preparation of Dimeric Analogues of Amiloride

## EXAMPLE 2

N-Cbz-1-(3,5-diamino-6-chloropyrazinoyl)-2-methyl-pseudothiourea (III)

1 (II, 494 mg, 1.27 mmol) was dissolved in a mixture of anhydrous DMF (10 mL); and triethylamine (3 mL) followed by treatment with N-(benzyloxycarbonyloxy) succinimide (470 mg, 1.7 mmol) dissolved in DMF (3 mL). The reaction mixture was stirred overnight at room temperature. After this time, the reaction mixture was concentrated under reduced pressure and the residue suspended in ethyl acetate (30 mL). Silica gel (25 g) was added to the solution and the solvent was evaporated to leave the silica gel impregnated with the crude product that was purified by flash chromatography on a FlashElute™ system from Elution Solution (P.O. Box 5147, Charlottesville, Virginia 22905) using a 90 g silica gel cartridge (eluent: hexanes, ethyl acetate = 1:2). The purified N-Cbz-1-(3,5-diamino-6-chloropyrazinoyl)-2-methyl-pseudothiourea (III) was obtained as a pale yellow solid: 416 mg (83% yield); <sup>1</sup>H NMR (360 MHz, DMSO-*d*<sub>6</sub>) δ 2.33 (s, 3H), 2.61 (s, 3H), 4.99 (s, 2H), 7.39 (m, 10H), 13.7 (s, 1H); API MS *m/z* = 395 [C<sub>15</sub>H<sub>15</sub>ClN<sub>6</sub>O<sub>3</sub>S + H]<sup>+</sup>; LC/MS (Method A) >99%, *t<sub>r</sub>* = 10.1 min.

## EXAMPLE 3

1,5-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]-3-oxa-pentane Dihydrobromide (Ia)

25 A solution of 1,5-diamino-3-oxa-pentane (IVa, 30 μL, 0.3 mmol) in dry DMF (100 μL) was added to intermediate III (226 mg, 0.6 mmol) and stirred in anhydrous DMF (10 mL). Triethylamine (480 μL, 3.4 mmol) in DMF (1 mL) and mercury(II) chloride (154 mg, 0.6 mmol) in DMF (100 μL) were added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was filtered through silica gel and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography on FlashElute™ system from Elution Solution using 90 g silica gel cartridge (eluent: ethyl acetate, hexanes = 7:1). The fractions were analyzed by LC/MS (Method B) and those fractions containing the desired

35

product were combined and concentrated to give 1,5-bis[(N-Cbz-3,5-diamino-6-chloropyrazinoyl)guanidino]-3-oxa-pentane (Va, 102 mg, 44% yield); LC/MS >99% (Method B); API MS  $m/z = 797$  [ $C_{32}H_{34}Cl_2N_{14}O_7 + H$ ]<sup>+</sup>.

Intermediate Va (50 mg) was dissolved in 30% HBr in acetic acid (10 mL) and the mixture was stirred for 2 d. The volume of the reaction mixture was reduced (to 4 mL) when a precipitate formed. Ethyl ether (10 mL) was added to the acetic acid/product mixture and the precipitate was collected by vacuum filtration, washed with additional ether, dried and then purified by preparative HPLC on Luna column [C18(2), 5 $\mu$ , 250 x 21.2 mm; mobile phase MeCN/water containing 0.1% TFA; gradient: 5% MeCN from the 0-2 min interval, increased from 5% - 40% MeCN from 2-10 min, 40% - 80% MeCN from 10-19 min, 40% - 80% MeCN from 19-23 min, 80% - 100% MeCN and 100% MeCN from 23-25 min.] Fractions containing the target compound were combined, concentrated under reduced pressure and the residue redissolved in 10% HBr and evaporated to dryness and washed with THF. The product Ia was obtained as a yellow powder: 18.9 mg (41% yield from V); <sup>1</sup>H NMR (360 MHz, DMF-*d*<sub>7</sub>)  $\delta$  3.80 (m, 4H), 3.88 (m, 4H), 7.51 (br s, 4H), 9.58 (m, 2H), 10.97 (s, 2H). API MS  $m/z = 529$  [ $C_{16}H_{22}Cl_2N_{14}O_3 + H$ ]<sup>+</sup>; HPLC (Method D) >99%,  $t_r = 6.72$  min.

#### EXAMPLE 4

##### 1,4-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidinol]butane dihydrobromide (Ib)

A solution of 1,4-diaminobutane (IVb, 24 mg, 0.3 mmol) in dry DMF (230  $\mu$ L) was added to III (213 mg, 0.54 mmol) in anhydrous DMF (10 mL), followed by addition of triethylamine (480  $\mu$ L, 3.4 mmol) in DMF (1 mL) and mercury(II) chloride (146 mg, 0.53 mmol) in DMF (600  $\mu$ L). The reaction mixture was stirred for 3 d at room temperature, then filtered through silica gel. The filtrate was concentrated under reduced pressure and the residue was dissolved in 30% HBr in acetic acid (20 mL) and stirred overnight at rt. The reaction mixture was poured into ethyl ether (150 mL) resulting in the formation of a precipitate that was isolated by vacuum filtration and washed with water (3 x 0.5 mL). The solid precipitate was purified by preparative HPLC on a Luna C18(2) column [5 $\mu$ , 250 x 21.2 mm; flow rate = 20 mL/min; mobile phase consists of MeCN/water containing 0.1% TFA; gradient: 10% MeCN from the 0-2 min interval, concentration of MeCN increased from 10% - 40%



from 2-10 min, 40% - 100% MeCN from 10-19 min, 100% MeCN from 19-23 min, MeCN decreased from 100% - 10% from 23-25 min]. Fractions containing the target compound were combined and concentrated under reduced pressure to give a residue that was redissolved in 10% HBr and evaporated to dryness and washed with ethyl ether to give **Ib** as a pale yellow solid: 19.4 mg (10.1% yield); <sup>1</sup>H NMR (360 MHz, DMSO-*d*<sub>6</sub>) δ 1.62 (br s, 4H), 7.43 (s, 4H), 8.77 (br s, 2H), 8.89 (br s, 2H), 9.24 (s, 2H), 10.48 (s, 2H); <sup>13</sup>C NMR (90 MHz, DMSO-*d*<sub>6</sub>) δ 24.8, 40.4, 108.9, 119.5, 153.1, 154.2, 155.9 and 165.1; API MS = 513 [C<sub>16</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>14</sub>O<sub>2</sub>+H]<sup>+</sup>; HPLC (Method D) >99%, *t*<sub>r</sub> 6.26 min.

10

### EXAMPLE 5

#### 1,5-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]hexane Dihydrobromide (Ic)

Compound **Ic** was prepared following the same procedure described for **Ib**.  
The Cbz-protected pseudothiourea **III** (226 mg, 0.6 mmol) and 1,6-diaminohexane (**IVc**, 34.9 mg, 0.3 mmol) reacted in the presence of triethylamine (480 μL, 3.4 mmol) and mercury(II) chloride (162.9 mg, 0.6 mmol) to give the crude intermediate **Vc**, which was treated with 30% HBr in acetic acid as previously described. The crude product was purified by preparative HPLC on a Luna C18(2) column [5μ, 250 x 21.2 mm; flow rate = 20 mL/min; mobile phase: MeCN/water (containing 0.1% TFA); gradient: 15% MeCN for 0-2 min interval, increase concentration of MeCN from 15% - 30% from 2-10 min, 30% - 50% MeCN from 10-19 min, 50% - 100% MeCN from 19-23 min, then decrease concentration MeCN from 100% - 15% from 23-25 min]. Fractions containing the target compound were combined and concentrated under reduced pressure to give a residue that was redissolved in 10% HBr and evaporated to dryness and washed with ethyl ether to give **Ic**: 28.8 mg (13.5% yield based on **III**); <sup>1</sup>H NMR (360 MHz, DMSO-*d*<sub>6</sub>) δ 1.38 (br s, 4H), 1.59 (br s, 4H), 3.38 (m, 2H), 7.44 (s, 4H), 8.75 (br s, 2H), 8.90 (br s, 2H), 9.19 (s, 2H) and 10.47 (s, 2H); <sup>13</sup>C NMR (90 MHz, DMSO-*d*<sub>6</sub>) δ 25.5, 27.5, 40.9, 108.9, 119.6, 153.1, 154.2, 155.8 and 165.1; API MS *m/z* = 541 [C<sub>18</sub>H<sub>26</sub>Cl<sub>2</sub>N<sub>14</sub>O<sub>2</sub>+H]<sup>+</sup>; HPLC (Method D) 95.2%, *t*<sub>r</sub> = 7.26 min.

## EXAMPLE 6

1, 3-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]xylyleneDihydrobromide (Id)

5 Compound Id was prepared following the same procedure described for Ib. Triethylamine (480  $\mu$ L, 3.4 mmol) and mercury(II) chloride (192 mg, 0.7 mmol) were added to a solution of Cbz-protected pseudothiourea III (280 mg, 0.7 mmol) and 1,3-xylylene diamine (IVd, 50 mg, 0.3 mmol) in DMF (30 mL). The reaction mixture was stirred at rt for 48 h and worked up the same as in the procedure for Ib and  
10 followed by treatment of the crude intermediate Vd with 30% HBr in acetic acid as previously described. The resulting crude product (yellow solid) was crystallized from methanol and further purified by preparative HPLC on a symmetry C8-column [7 $\mu$ , 200 x 40 mm; flow rate = 40 mL/min; mobile phase: MeCN/water (containing 0.1% TFA); gradient: concentration of MeCN 5% for 0-2 min interval, then increased  
15 from 5% - 20% MeCN from 2-10 min, 20% - 60% MeCN from 10-30 min, 60% - 100% MeCN from 30-33 min and concentration decreased from 100% - 5% MeCN from 33-35 min]. Product isolation and further treatment with HBr as previously described gave the product Id as pale yellow solid: 31.2 mg (12.1% yield from III); <sup>1</sup>H NMR (360 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  4.60 (d, *J* = 5.2 Hz, 4H), 7.40-7.42 (m, 7h), 9.03 (br  
20 s, 4H), 9.61 (s, 2H) and 10.59 (s, 2H); API MS *m/z* = 561 [C<sub>20</sub>H<sub>22</sub>Cl<sub>2</sub>N<sub>14</sub>O<sub>2</sub>+H]<sup>+</sup>; HPLC (Method E) 97.3%, *t<sub>r</sub>* = 5.5 min.

## EXAMPLE 7

1,5-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]pentane Dihydrobromide (Ie)

25 Compound Ie was prepared following the same procedure described for Ib. The Cbz-protected pseudothiourea III (280 mg, 0.7 mmol) and 1,5-diaminopentane (IVe, 37 mg, 0.35 mmol) were reacted in the presence of triethylamine (480  $\mu$ L, 3.4 mmol) and mercury(II) chloride (192 mg, 0.7 mmol). The reaction mixture was stirred at rt for 24 h and worked up the same as in the procedure for Ib and the  
30 resulting crude intermediate Ve was treated with 30% HBr in acetic acid for 24 h as previously described. The reaction mixture was poured into ethyl ether (200 mL), the precipitate was collected by filtration, washed with ether, THF and then crystallized twice from 12% HBr to give crude Ie (117 mg, 87% purity, 47% yield from III) as a

yellow solid. A portion of this material (78 mg) was crystallized again from 12% HBr to give **Ie** as a pale yellow solid: 32 mg (12.8% yield from **III**);  $^1\text{H}$  NMR (360 MHz, DMSO- $d_6$ )  $\delta$  1.39 (m, 2H), 1.61 (m, 4H), 3.31 (m, 4H), 7.44 (br s, 4H), 8.72 (br s, 2H), 8.90 (br s, 2H), 9.20 (s, 2H) and 10.49 (s, 2H);  $^{13}\text{C}$  NMR (90 MHz, DMSO- $d_6$ )  $\delta$  23.1, 27.3, 40.8, 109.0, 119.7, 153.1, 154.2, 155.9 and 165.2; API MS  $m/z$  = 527  
5  $[\text{C}_{17}\text{H}_{24}\text{Cl}_2\text{N}_{14}\text{O}_2+\text{H}]^+$ ; HPLC (Method E) 95.3%,  $t_r$  = 5.72 min.

### EXAMPLE 8

#### 1,5-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]pentane Dihydrochloride (**Ie** • 2 HCl)

10

The combined mother liquors from the crystallization of **Ie** were treated with powder KOH until the solution reached pH=11. The precipitate that formed was collected by vacuum filtration, washed with water, and recrystallized twice from 10% aqueous HCL to give **Ie** • 2HCl as a pale yellow solid: 27.3 mg (13% yield from  
15 **III**);  $^1\text{H}$  NMR (360 MHz, DMSO- $d_6$ )  $\delta$  1.40 (m, 2H), 1.61 (m, 4H), 3.34 (m, 4H), 7.41 (br s, 4H), 8.80 (br s, 2H), 8.93 (br s, 2H), 9.29 (s, 2H) and 10.52 (s, 2H);  $^{13}\text{C}$  NMR (90 MHz DMSO- $d_6$ )  $\delta$  23.1, 27.3, 40.8, 109.0, 119.7, 153.2, 154.2, 155.9 and 165.2; API MS  $m/z$  = 527  $[\text{C}_{17}\text{H}_{24}\text{Cl}_2\text{N}_{14}\text{O}_2+\text{H}]^+$ ; HPLC (Method E) 95.2%,  $t_r$  = 5.78 min.

20

### EXAMPLE 9

#### 1,4-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]xylylene Dihydrobromide (**If**)

Compound **If** was prepared following the same procedure described for **Ib**. The Cbz-protected pseudothiourea **III** (280 mg, 0.7 mmol) and 1,4-xylylenediamine (**IVf**, 50 mg, 0.30 mmol) were reacted in the presence of triethylamine (480  $\mu\text{L}$ , 3.4  
25 mmol) and mercury(II) chloride (192 mg, 0.7 mmol). The reaction mixture was stirred at rt for 4 d and then it was filtered through silica gel and concentrated under reduced pressure. The residue was suspended in anhydrous DMF (10 mL) and treated with tetrabutylammonium borohydride (50 mg, 0.17 mmol) in DMF (1 mL) and stirred for 15 min at rt to get rid of residual mercury(II) chloride. The reaction  
30 mixture was filtered through silica gel and concentrated to give a residue (**Vf**) that was treated with 30% HBr in acetic acid (20 mL) for 7 d at rt and 1 d at 45 °C. The reaction mixture was poured into ether (200 mL) and the solid that precipitated was collected by filtration, washed with ether, THF and crystallized twice from methanol

to give a pale yellow solid: 74 mg (31% yield from **III**);  $^1\text{H}$  NMR (360 MHz, DMSO- $d_6$ )  $\delta$  4.60 (d,  $J = 4.3$  Hz, 4H), 7.45 (s, 4H), 8.91 (br s, 2H), 8.99 (br s, 2H), 9.60 (s, 2H) and 10.56 (s, 2H);  $^{13}\text{C}$  NMR (90 MHz, DMSO- $d_6$ )  $\delta$  44.1, 109.1, 119.7, 128.1, 135.4, 153.3, 154.3, 156.0 and 165.3; API MS  $m/z = 561$  [ $\text{C}_{20}\text{H}_{22}\text{Cl}_2\text{N}_{14}\text{O}_2 + \text{H}$ ] $^+$ ;  
5 HPLC (Method E) 95.7%,  $t_r = 6.31$  min.

### EXAMPLE 10

#### 1,8-Bis[(3,5-diamino-6-chloropyrazinoyl)guanidino]-3,6-dioxaoctane Dihydrochloride (Ig)

10

A solution of 2,2'-(ethylenedioxy)bis(ethylamine) (**IVg**, 45 mg, 0.3 mmol) in dry DMF (100  $\mu\text{L}$  was added to **III** (240 mg, 0.7 mmol) in dry DMF (30 mL), followed by addition of triethylamine (480  $\mu\text{L}$ , 3.4 mmol) in DMF (1 mL) and mercury(II) chloride (165 mg, 0.6 mmol) in DMF (600  $\mu\text{L}$ ). The reaction mixture  
15 was stirred 16 h at room temperature, then additional **III** (20 mg) was added and reaction mixture was stirred an additional 8 h at 40  $^\circ\text{C}$ . The reaction mixture was cooled to room temperature and treated with tetrabutylammonium borohydride (50 mg, 0.17 mmol) in DMF (1 mL) with stirring for 15 min at room temperature. The reaction mixture was filtered through silica gel, concentrated under reduced pressure  
20 to give a solid residue. This was dissolved in 30% HBr in acetic acid (20 mL) and stirred for 8 h at 40  $^\circ\text{C}$ , then poured into ether (200 mL). The resulting precipitate was collected by filtration and washed with ether. The solid was dissolved in water (25 mL), the solution filtered, and the filtrate concentrated under reduced pressure. The resulting residue was dissolved again in minimal 10% HBr and powdered NaOH is  
25 added to pH=11. A precipitate formed and was collected by filtration, washed with water and dried to give the free base (98 mg, 56% yield). A portion of this material (58 mg) was dissolved in 10% HCl and then concentrated under reduced pressure. The residue is washed with ether and dried to give **Ig** as a pale yellow solid: 64 mg (32% yield from **III**);  $^1\text{H}$  NMR (360 MHz, DMSO- $d_6$ )  $\delta$  3.56 (br s, 4H), 3.63 (br s, 8H), 7.4 (br s, 4H), 9.09 (br s, 4H), 9.52 (br s, 2H) and 10.70 (s, 2H);  $^{13}\text{C}$  NMR (90  
30 MHz, DMSO- $d_6$ )  $\delta$  41.1, 67.7, 69.5, 108.9, 119.7, 153.3, 154.2, 155.8 and 165.3; API MS  $m/z = 573$  [ $\text{C}_{18}\text{H}_{26}\text{Cl}_2\text{N}_{14}\text{O}_4 + \text{H}$ ] $^+$ ; LC (Method C) 97.6%,  $t_r = 4.23$ .

**EXAMPLE 11**  
**Potency of Dimeric Compounds**

Two pharmacologic assays were used to determine the relative potency of the dimers described herein. The first assay examined the expression of the subunits  $\alpha$ ,  $\beta$ , and  $\gamma$  of recombinant apical membrane epithelial  $\text{Na}^+$  channel (or "rENaC") in *Xenopus* oocyte, as follows: cRNAs for all three ENaC subunits were injected into oocytes via conventional microinjection techniques. After two to three days, two electrode voltage clamp protocols were used to measure ENaC-mediated  $\text{Na}^+$  currents. Test compounds were assayed using cumulative drug addition protocols known in the art. Single oocytes were used for single compounds. Compounds tested were then compared to dose-effect relationships for amiloride and benzamil in the same batch of injected eggs.

In the second potency assay, airway epithelial monolayers mounted were in Ussing chambers: The principal assay consisted of tests of luminal drug inhibition of airway epithelial  $\text{Na}^+$  currents. Cells obtained from freshly excised human or dog airways were seeded onto SNAP-well Inserts (CoStar), cultured under air-liquid (ALI) conditions in hormonally defined media. The cells were assayed for  $\text{Na}^+$  transport activity while bathed in Krebs bicarbonate Ringer (KBR) in the Ussing chambers under voltage clamp conditions. All test drug additions were to the mucosal bath with half-log dose addition protocols ( $10^{-11}\text{M}$  -  $10^{-5}\text{M}$ ). All drugs were made in standard stocks of  $10^{-2}\text{M}$  drug in DMSO. Eight preparations were typically run in parallel; two preparations/run were routinely used to assay amiloride and benzamil. After the maximal concentration ( $10^{-4}\text{M}$ ) was administered, the luminal bath was exchanged three times with fresh KBR solution, which was defined as the "wash-out" effect. All data from the voltage clamps were collected via a computer interface and analyzed off-line.

Dose-effect relationships for all compounds were considered and analyzed by the Prism 3.0 program. The  $\text{IC}_{50}$ , maximal effective concentrations, and percent washout were calculated and compared to those of amiloride and benzamil as reference compounds.

### EXAMPLE 13

#### Absorption Assays

Compounds useful in the present invention preferably have the characteristics of high potency and non- or decreased absorbability into mucosal surfaces. Two  
5 pharmacologic assays were employed to test the absorption of compounds illustrated in TABLE 1.

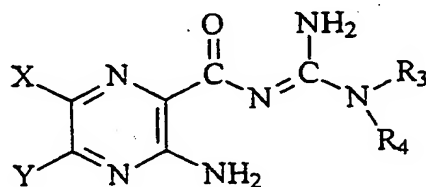
The first assay is referred to an assay of reversibility. Empirically, the percent of wash-out correlates with cellular uptake. The relationship is complex because reversibility is also a function of potency. However, reversibility is a quick and  
10 simple screen. An example of the information obtained by such as assay is shown in FIG. 1. The compounds that "reverse" best in this assay referenced to benзамил were also the least absorbed in the confocal assay, as described below.

The second assay utilizes confocal microscopy of amiloride congener uptake: Virtually all amiloride-like molecules fluoresce in UV range. This property of these  
15 molecules was used to directly measure cellular uptake, using a x-z confocal microscope (Leica). As an example of the results obtained by this assay are shown in FIG. 2. In the experiment shown in FIG. 2, equimolar concentrations of amiloride and compounds of rapid (benзамил) and very rapid uptake (phenamil) were placed on the apical surface of airway cultures on the stage of the confocal microscope. Serial  
20 x-z images were obtained with time and the magnitude of fluorescence accumulating in the cell compartment quantitated and plotted. The assay was subsequently optimized to test for compounds that absorbed into cells less rapidly than amiloride. Two compounds from the synthesis series described above (CF-509 and CF-519) appear to fulfil this criterion. Compounds that were equipotent or greater with  
25 amiloride were tested for wash-out as described above. However, because wash-out may reflect both potency and cell uptake, the rate of accumulation of fluorescence (indexed to the specific fluorescence/emission spectrum of each compound) in the cell compartment as a function of time was also routinely measured. The relative cellular uptake of each test compound was then compared to the reference compounds  
30 (amiloride, benзамил) as for potency assays.

The foregoing is illustrative of the present invention, and is not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be included therein.

THAT WHICH IS CLAIMED IS:

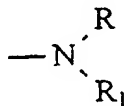
1. A compound of the formula  $P_1-L-P_2$ , wherein:  
 $P_1$  is a pyrazinoylguanidine sodium channel blocker;  
 $L$  is a linking group; and  
 $P_2$  is selected from the group consisting of pyrazinoylguanidine sodium  
 channel blockers and  $P2Y_2$  receptor agonists;  
 or a pharmaceutically acceptable salt thereof.
2. A compound according to claim 1, wherein  $P_2$  is a pyrazinoylguanidine  
 sodium channel blocker.
3. A compound according to claim 2, wherein:  
 $P_1$  and  $P_2$  are each independently selected from the group consisting of  
 compounds of the formula:



wherein:

$X$  is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, Z-thio and Z-sulfonyl wherein  $Z$  is selected from the group consisting of loweralkyl, oxyalkyl, and phenyl-loweralkyl.;

$Y$  is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, and amino having the structure:



wherein:

$R$  is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-

loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

$R_1$  is selected from the group consisting of hydrogen, loweralkyl,  
5 loweralkenyl, and additionally;

$R$  and  $R_1$  can be joined to form a lower alkylene;

$R_2$  is selected from the group consisting of hydrogen and loweralkyl;

$R_3$  and  $R_4$  are independently selected from the group consisting of hydrogen, loweralkyl, hydroxy-loweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-  
10 (alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl; and

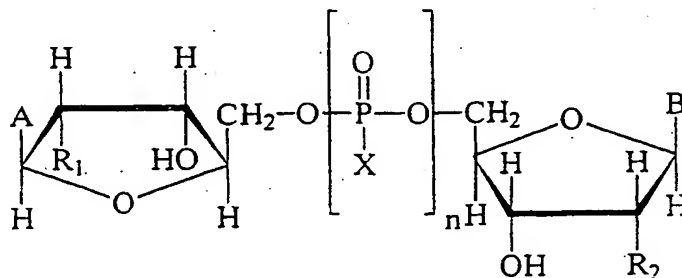
$L$  is selected from the group consisting of loweralkyl, hydroxy-loweralkyl,  
15 phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl.

20

4. A compound according to claim 1, wherein said  $P_2$  is a nucleotide or dinucleotide  $P_2Y_2$  receptor agonist.

5. A compound according to claim 4, wherein  $P_2$  is a dinucleotide having the  
25 formula:

30



wherein:

A and B are each independently a purine or pyrimidine base;



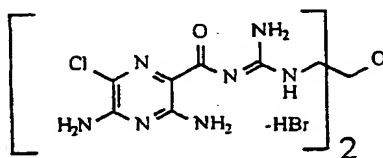
$R_1$  and  $R_2$  are each independently selected from the group consisting of H or OH; and

$n$  is from 1 to 6;

with said dinucleotide covalently joined to L by covalent attachment to A or B or

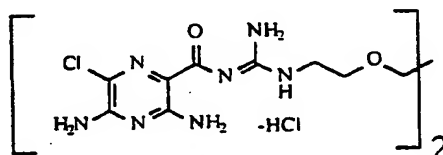
5 covalent attachment to the ribose or deoxyribose ring to which A and B are joined.

6. A compound of Claim 1, wherein the compound has the structure:



10

7. A compound of Claim 1, wherein the compound has the structure:



8. A compound of Claim 1, wherein  $P_1$  is selected from the group consisting of amiloride, benzamil and phenamil.

15

9. A pharmaceutical formulation comprising a compound according to Claim 1 in a pharmaceutically acceptable carrier solution.

20

10. A method of treating a mucosal surface in a subject in need thereof, comprising administering to said subject a compound according to Claim 1 in an amount effective to treat said mucosal surface.

11. A method according to Claim 10, wherein said mucosal surface is an airway mucosal surface.

25

12. A method according to claim 10, wherein said mucosal surface is a gastrointestinal mucosal surface.

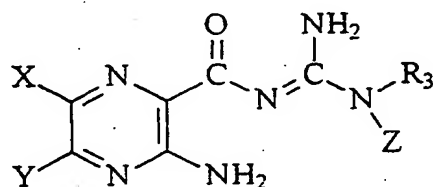
13. A method of treating a mucosal surface in a subject in need thereof,  
5 comprising administering to said subject, in an amount effective to treat a mucosal surface, a compound comprising a covalent conjugate of a pyrazinoylguanidine sodium channel blocker and a non-absorbable carrier moiety.

14. A method according to Claim 13, subject to the proviso that said mucosal  
10 surface is not a nasal mucosal surface.

15. A method according to Claim 13, wherein said mucosal surface is an airway mucosal surface.

16. A method according to Claim 13, wherein said mucosal surface is a  
15 gastrointestinal mucosal surface

17. A method according to claim 13, wherein said conjugate has the formula:  
formula:

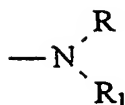


20

wherein:

X is selected from the group consisting of chloro, bromo, iodo, loweralkyl, lower-cycloalkyl having from 3 to 7 carbons, phenyl, chlorophenyl, bromophenyl, Z'-thio and Z'-sulfonyl wherein Z' is selected from the group consisting of loweralkyl  
25 and phenyl-loweralkyl.;

Y is selected from the group consisting of hydroxyl, mercapto, loweralkyloxy, loweralkylthio, chloro, loweralkyl, lowercycloalkyl having from 3 to 6 carbons, phenyl, amino having the structure:



wherein:

R is selected from the group consisting of hydrogen, amino, amidino, lower-cycloalkyl having 3 to 6 carbon atoms, loweralkyl, hydroxyloweralkyl, halo-loweralkyl, lower-(cycloalkylalkyl) having 3 to 6 carbons in the ring, phenyl-loweralkyl, lower-(alkylaminoalkyl), lower-alkenyl, phenyl, halophenyl, and lower-alkylphenyl;

R<sub>1</sub> is selected from the group consisting of hydrogen, loweralkyl, loweralkenyl, and additionally;

R and R<sub>1</sub> can be joined to form a lower alkylene;

R<sub>2</sub> is selected from the group consisting of hydrogen and loweralkyl;

R<sub>3</sub> is selected from the group consisting of hydrogen, loweralkyl, hydroxyloweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl; and

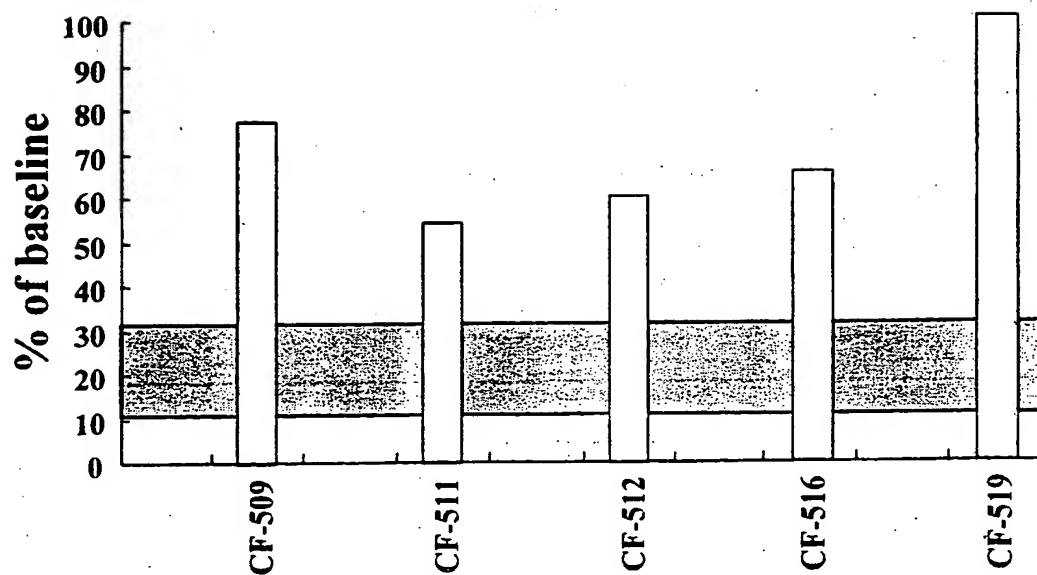
L is selected from the group consisting of loweralkyl, hydroxy-loweralkyl, phenyl-loweralkyl, (halophenyl)-loweralkyl, lower-(alkylphenylalkyl), (loweralkoxyphenyl)-loweralkyl, naphthyl-loweralkyl, (octahydro-1-azocinyl)-loweralkyl, pyridyl-loweralkyl, and loweralkyl radicals linked to produce with the nitrogen atom to which they are attached a 1-pyrrolidinyl, piperidino, morpholino, and a 4-loweralkyl-piperazinyl group, and phenyl; and

Z is a non-absorbable carrier moiety covalently linked to the adjacent nitrogen atom;

or a pharmaceutically acceptable salt thereof.

1/2

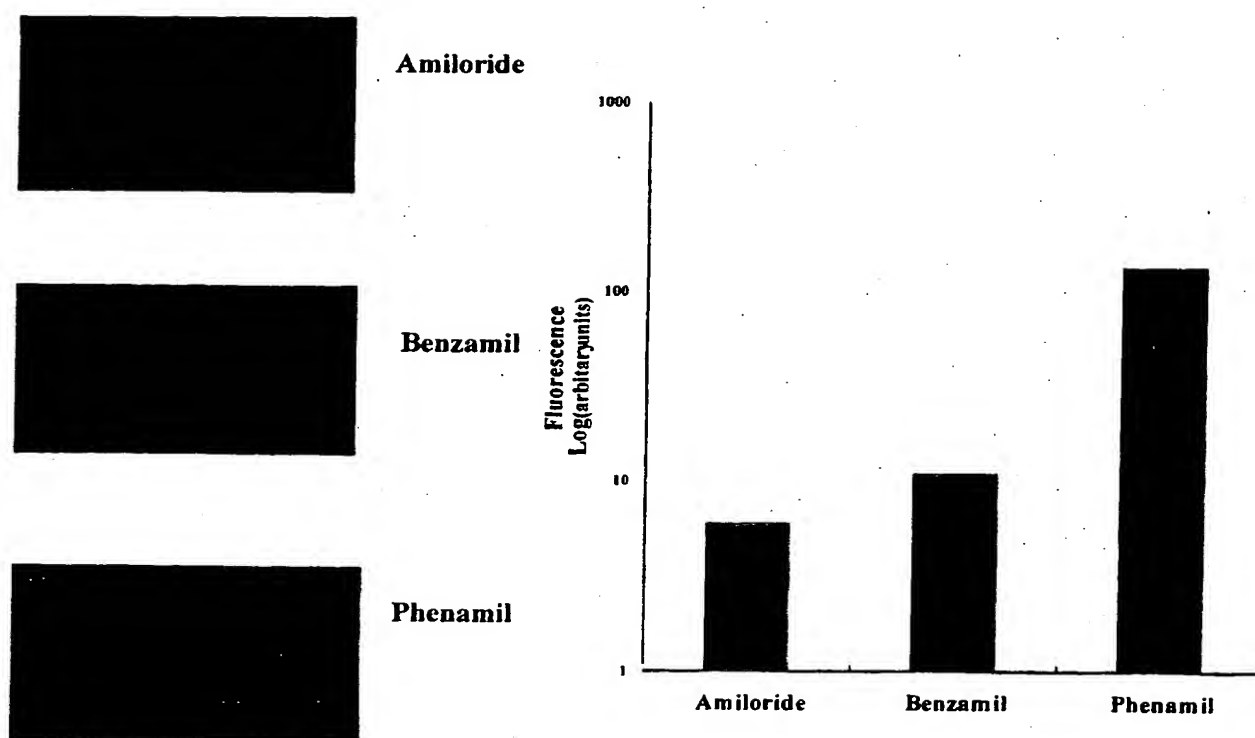
Figure 1.



BEST AVAILABLE COPY

2/2

Figure 2



BEST AVAILABLE COPY

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/19775

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D241/26 C07H19/10 C07H19/207 A61K31/495 A61K31/70  
A61P11/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D C07H A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 18385 A (THE UNIVERSITY OF NORTH CAROLINA) 20 June 1996 (1996-06-20) cited in the application the whole document	1-17
Y	WO 94 08593 A (THE UNIVERSITY OF NORTH CAROLINA) 28 April 1994 (1994-04-28) cited in the application the whole document	1-17
Y	WO 96 40059 A (THE UNIVERSITY OF NORTH CAROLINA) 19 December 1996 (1996-12-19) cited in the application the whole document	1-17
	-/-	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*S\* document member of the same patent family

Date of the actual completion of the international search

20 November 2000

Date of mailing of the international search report

29/11/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Beslier, L

# INTERNATIONAL SEARCH REPORT

Intel. Jnal Application No

PCT/US 00/19775

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 837 861 A (WILLIAM PENDERGAST) 17 November 1998 (1998-11-17) cited in the application the whole document ----	1-17
A	US 3 313 813 A (EDWARD J. CRAGOE) 11 April 1967 (1967-04-11) ----	
P,X	WO 00 23023 A (THE UNIVERSITY OF NORTH CAROLINA) 27 April 2000 (2000-04-27) the whole document -----	13-17

BEST AVAILABLE COPY

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. Appl. No.

PCT/US 00/19775

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9618385 A	20-06-1996	US 5656256 A AU 4420496 A CA 2207585 A EP 0793483 A US 5876700 A	12-08-1997 03-07-1996 20-06-1996 10-09-1997 02-03-1999
WO 9408593 A	28-04-1994	US 5292498 A AU 688258 B AU 2872592 A EP 0663830 A JP 8502078 T NO 951340 A	08-03-1994 12-03-1998 09-05-1994 26-07-1995 05-03-1996 02-06-1995
WO 9640059 A	19-12-1996	US 5635160 A AU 696845 B AU 6176496 A BR 9609063 A CA 2223894 A EP 0831777 A JP 11506790 T NO 975632 A US 5935555 A	03-06-1997 17-09-1998 30-12-1996 26-01-1999 19-12-1996 01-04-1998 15-06-1999 03-02-1998 10-08-1999
US 5837861 A	17-11-1998	AU 6324298 A BR 9807169 A EP 0981534 A NO 993776 A WO 9834942 A ZA 9801073 A	26-08-1998 06-06-2000 01-03-2000 06-10-1999 13-08-1998 19-02-1999
US 3313813 A	11-04-1967	BE 639386 A CH 465617 A CH 481116 A DE 1470053 A DE 1695443 A DE 1795438 B DK 114901 B DK 116943 B DK 114978 B FR 1563612 A GB 1066855 A MY 2669 A NL 142413 B SE 321230 B US 3781430 A DK 115403 B DK 114557 B FI 47102 B NL 299931 A	15-11-1969 26-06-1969 08-04-1971 06-09-1973 18-08-1969 02-03-1970 25-08-1969 18-04-1969 31-12-1969 17-06-1974 02-03-1970 25-12-1973 06-10-1969 14-07-1969 31-05-1973
WO 0023023 A	27-04-2000	AU 1214100 A	08-05-2000

BEST AVAILABLE COPY



CORRECTED VERSION

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
25 January 2001 (25.01.2001)

PCT

(10) International Publication Number  
**WO 01/05773 A1**

(51) International Patent Classification<sup>7</sup>: C07D 241/26,  
C07H 19/10, 19/207, A61K 31/495, 31/70, A61P 11/12

(21) International Application Number: PCT/US00/19775

(22) International Filing Date: 19 July 2000 (19.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/144,479 19 July 1999 (19.07.1999) US  
09/618,978 19 July 2000 (19.07.2000) US

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:  
— With international search report.

(48) Date of publication of this corrected version:  
14 June 2001

(71) Applicant (*for all designated States except US*): THE UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL [US/US]; 308 Bynum Hall, Campus Box 4105, Chapel Hill, NC 27599-4105 (US).

(71) Applicant and  
(72) Inventor: BOUCHER, Richard, C., Jr. [US/US]; 735 Gimghoul Road, Chapel Hill, NC 27514 (US).

(74) Agent: MYERS BIGEL SIBLEY SAJOVEC, P.A.; Post Office Box 37428, Raleigh, NC 27627 (US).

(15) Information about Correction:  
see PCT Gazette No. 24/2001 of 14 June 2001, Section II

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: CONJUGATES OF SODIUM CHANNEL BLOCKERS AND METHODS OF USING THE SAME

(57) Abstract: Compounds of the general formula P<sub>1</sub>-L-P<sub>2</sub>; wherein "P<sub>1</sub>" is a pyrazinoylguanidine sodium channel blocker, "L" is a linking group, and "P<sub>2</sub>" is either (i) a pyrazinoylguanidine sodium channel blocker or (ii) a P2Y<sub>2</sub> receptor agonist, are disclosed. Pharmaceutical formulations containing the same and methods of use thereof to hydrate mucosal surfaces such as airway mucosal surfaces are also disclosed.

WO 01/05773 A1